b) irradiating the treated germ cell with a high-energy beam;

c) subjecting the irradiated germ cell to artificial fertilization to induce mutagenesis in an embryo;

d) expressing a mutated gene; and

e) examining the correlation between the mutated gene and the mutant phenotype.

A1 cont'd

19. (new) The method of claim 18, wherein the germ cell is sperm.

20. (new) The method of claim 18 or 19 wherein the psoralen derivative is 4,5',8-trimethylpsoralen.

21. (new) The method of claim 18 or 19 wherein the psoralen derivative is 4,5',8-trimethylpsoralen and the vertebrate animal is zebrafish.

22. (new) The method of claim 18 or 19 wherein the mutagenesis is introduced into a region containing a pyrimidine base.

Kindly cancel claims 1-7 without prejudice or disclaimer.

REMARKS

New claims 8-22 have been introduced and claims 1-7 have been cancelled without prejudice or disclaimer. No new matter has been added by virtue of these amendments. Support for such amendments can be found throughout the specification and in the original claims of the application. For example, see page 7, lines 18-23 and page 14, lines 19-24.

Claims 2, 4, and 5 were rejected under 35 U.S.C. §112, second paragraph, as being unclear and indefinite.

Claims 1-7 were rejected under 35 U.S.C. §101 because the claimed recitation of a use does not set forth a proper step involved with the process of use.

It is believed that the amendments submitted herein obviate the rejections under §101 and §112, second paragraph. See, for example, new claims 8, 10, 11, 13, 15, 16, 18, 20, and 21.

Withdrawal of the noted rejection is thus requested.

Claims 1,2, and 5-7 were rejected under 35 U.S.C §102 (b) as being anticipated by Glazer et al. (WO 95/01364).

Claim 3 was rejected under 35 U.S.C. §103(a) as being unpatentable over Stuart et al. (*Development*, Vol. 107, p. 577-584, 1990) in view of Glazer et al. (WO 95/01364).

For the sake of brevity, these two rejections are addressed in combination. Such a combined response is considered appropriate because each rejection relies on the Glazer citation.

The rejections are respectfully traversed.

The present invention provides methods for the mutagenesis of a gene of a vertebrate animal and a method for the preparation of a mutated gene of a vertebrate animal, the methods comprising the steps of:

a) treating a germ cell of the vertebrate animal with a psoralen derivative;

b) irradiating the treated germ cell with a high-energy beam; and

c) subjecting the irradiated germ cell to artificial fertilization to induce mutagenesis in an embryo.

Moreover, Applicants have surprisingly discovered that a psoralen derivative can functionally crosslink nucleic acids such as DNA when irradiated by a high-energy irradiation source, for example, a UV or X-ray radiation source. The region of DNA defined by the site of psoralen derivative induced crosslinking can be deleted. See, for example, page 4, lines 18-23. Applicants provide preferred psoralen derivatives at pages 5 and 6 of the specification.

As the office action is understood, the Examiner asserts that Glazer et al (WO 95/01364) teaches a mutagenic oligonucleotide comprising trimethylpsoralen and methods for the use thereof.

Glazer merely a site-directed mutagenesis of a gene of interest using a mutagenic oligonucleotide to form a DNA triplex, as clearly described, for example, in the first three lines of "Background of the Invention," page 3 lines 35-37 and Claim 1. Moreover, the mutagenic oligonucleotide used in the site-directed mutagenesis of Glazer et al consists of a single-stranded oligonucleotide that forms a triple-stranded nucleic acid molecule with a target region of a particular double-stranded nucleic acid molecule, and a mutagen such as psoralen that is incorporated into the single-stranded oligonucleotide. Applicants note that the Glazer methods require genetic information in order to engineer a specific oligonucleotides which is capable of triplex formation with a specified DNA sequence.

As Glazer is understood, the mutagenic agent used for mutatgenesis is a single-stranded oligonucleotide having a mutagen coupled thereto which is capable of hybridization to a chosen site in the target region of a gene to form a triple stranded DNA with the DNA in the chosen site. Moreover, the use of a oligonucleotide with is capable of binding to a specified DNA sequence is an essential feature of the mutagenesis methods of Glazer. There is neither disclosure nor suggestion in Glazer of using a psoralen derivative such as 4,5',8-trimethylpsoralen as a mutagenic agent.

The disclosure of Stuart et al. is insufficient to overcome the deficiencies of Glazer. As cited, Stuart teaches the use of zebrafish as simple vertebrate animal models in transgenic studies and teaches that production, maintenance and analysis of mutant zebrafish cell lines are possible because of zebrafish have a short generational time period.

The methods of the present invention would not have been obvious to one skilled in the art from any combination of Glazer and Stuart. Moreover, the present invention would not have

been obvious by using for the mutagenic methods of Glazer using an mutagenic oligonucleotide to form a DNA triplex in a zebrafish subject as recited by Stuart

In contrast, the mutagenesis method of the present invention includes the use of a psoralen derivative to induce mutation by treating a germ cell with the psoralen derivative, irradiating the treated cell which typically induces mutation by deleting portions of the DNA sequence and inducing mutation in an embryo through artificial fertilization. Unlike Glazer where the mutagenic oligonucleotide is sequence specific, the mutagenesis methods of the present invention are not site-specific, e.g., mutagenesis methods of the invention are not based on a triple-stranded DNA formation to induce mutation and do not require genetic information to effect mutagenesis.

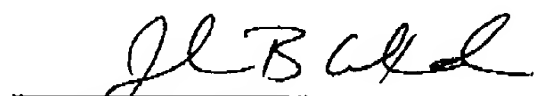
No combination of Glazer and Stuart taken in separately or in combination would motivate one skilled in the art to the subject matter of the present invention. Applicants respectfully submit that the present invention would not have been anticipated by or obvious over Glazer et al. optionally in view of Stuart et al.

Reconsideration and withdrawal of the rejection of the noted claims are thus requested.

It is believed the application is in condition for immediate allowance, which action is earnestly solicited.

Respectfully submitted,

December 29, 2001



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VERSION WITH CHANGES MARKED

(Additions are underlined; deletions are bracketed.)

8. (new) A mutagenesis method of a gene of a vertebrate animal, comprising the steps of:

a) treating a germ cell of the vertebrate animal with a psoralen derivative;

b) irradiating the germ cell with a high energy beam; and

c) subjecting the irradiated germ cell to artificial fertilization to induce mutagenesis in an embryo.

9. (new) A method according to claim 8, wherein the germ cell is sperm.

10. (new) The method of claim 8 or 9 wherein the psoralen derivative is 4,5',8-trimethylpsoralen.

11. (new) The method of claim 8 or 9 wherein the psoralen derivative is 4,5',8-trimethylpsoralen and the vertebrate animal is zebrafish.

12. (new) The method of claim 8 or 9 wherein the mutagenesis is introduced into a region containing a pyrimidine base.

13. (new) A method for preparation of a mutated gene of a vertebrate animal, comprising the steps of:

a) treating a germ cell of the vertebrate animal with a psoralen derivative;

b) irradiating the treated germ cell with a high-energy beam; and

c) subjecting the irradiated germ cell to artificial fertilization to induce mutagenesis in an embryo.

14. (new) The method of claim 13, wherein the germ cell is sperm.

15. (new) The method of claim 13 or 14 wherein the psoralen derivative is 4,5',8-trimethylpsoralen.

16. (new) The method of claim 13 or 14 wherein the psoralen derivative is 4,5',8-trimethylpsoralen and the vertebrate animal is zebrafish.

17. (new) The method of claim 13 or 14 wherein the mutagenesis is introduced into a region containing a pyrimidine base.

18. (new) A method for analyzing the function of a gene of a vertebrate animal, comprising the steps of:

a) treating a germ cell of the vertebrate animal with a psoralen derivative;

b) irradiating the treated germ cell with a high-energy beam;

c) subjecting the irradiated germ cell to artificial fertilization to induce mutagenesis in an embryo;

d) expressing a mutated gene; and

e) examining the correlation between the mutated gene and the mutant phenotype.

19. (new) The method of claim 18, wherein the germ cell is sperm.

20. (new) The method of claim 18 or 19 wherein the psoralen derivative is 4,5',8-trimethylpsoralen.

21. (new) The method of claim 18 or 19 wherein the psoralen derivative is 4,5',8-trimethylpsoralen and the vertebrate animal is zebrafish.

22. (new) The method of claim 18 or 19 wherein the mutagenesis is introduced into a region containing a pyrimidine base.